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USPT	11 same hydroxyapatite same HPLC	3	<u>L2</u>
USPT	(separat\$ or isolat\$ or purif\$) same nucleic	17351	<u>L1</u>

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L5: Entry 1 of 17

File: USPT

May 22, 2001

DOCUMENT-IDENTIFIER: US 6235503 B1

TITLE: Procedure for subtractive hybridization and difference analysis

BSPR:

An adaptation of RDA called cDNA-RDA has recently been described, Hubank & Schatz Nucleic Acids Research 22: 5640-5648 (1994), in which two populations of cDNA are compared for the presence a cDNA fragment representing either a mRNA unique to one of the two populations or a mRNA that is differentially expressed in the two populations. cDNA-RDA differs from the original RDA protocol in the following respects. 1) Since the complexity of the mRNA population of a typical mammalian cell is only .about.1-2% of genome complexity, generation of a representation is not required for the practice of cDNA-RDA. Hence, a more complete analysis of differences can be obtained in a single experiment. 2) Amplification of fragments already known to differ between the two populations can be minimized by addition of such fragments to the driver. 3) Amplification of fragments representing mRNAs present at different levels in the two populations (rather than absent in one population) can be achieved by depleting the populations of low-abundance sequences (by hybridization to low C.sub.o t) prior to amplification, and lowering the ratio of driver to tester during hybridizations subsequent to the generation of the first difference product. This effectively converts an up-regulated sequence into a unique sequence, for the purposes of the assay. A limitation of cDNA-RDA is the inability to detect differences due to point mutations, small deletions or small insertions, unless they affect a particular restriction enzyme recognition site. cDNA-RDA has been used to detect transcripts of a transfected gene in cultured cells and to clone cDNAs representing genes whose transcription is up-regulated in response to an environmental stimulus.